CAMPHANYLIDENE AND PHENYLALKYL INOSITOL POLYPHOSPHATE COMPOUNDS, COMPOSITIONS, AND METHODS OF THEIR USE

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Field of the Invention

This invention relates to new camphanylidene and phenyl alkyl inositol polyphosphate derivatives that modulate the absorption of sodium ions in epithelial cells and the upregulation of inducible nitric oxide synthase (iNOS) in macrophages. This

10 invention also relates to pharmaceutical compositions containing the compounds and to the use of the compounds and compositions, alone or in combination with other pharmaceutically active agents. The present invention also relates to methods for regulating the epithelial sodium channel (ENaC) and/or iNOS using effective camphanylidene and/or phenyl alkyl inositol polyphosphate compounds, alone or in

15 combination with other therapeutic agents, such as for treating pathological conditions related to cystic fibrosis, regulating fluid retention, regulating blood pressure in humans, treating inflammatory conditions, treating Alzheimer's disease, treating diabetes, treating pathological effects of ionizing radiation, and treating hyperproliferative disorders such as tumors, cancer, schleroderma, and hyperproliferative skin diseases such as psoriasis.

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Background of the Invention

Cystic fibrosis (CF) is the most common genetic disorder and the largest genetic killer of children. One in twenty Caucasians carries a defective CF gene, which, when coupled with a spouse who is also a carrier can result in offspring afflicted with CF. An autosomal, recessive disorder, one in 3,000 children born in the United States and Europe

25 inherit CF. Children live for varying periods of time, but the average has been extended from a couple of years early in this century to a current life expectancy of 30 years. Over 70,000 patients have been identified with Cystic Fibrosis worldwide. This translates into over 30,000 individuals with the disease in the United States with another 30,000 who have been identified with the disorder in Europe. As current treatment strategies prolong

30 the average lifespan, the number of CF patients is expected to rise. Patients with CF typically incur medical costs ranging from $15,000 to $55,000 annually.

The disease causes abnormally viscous mucous secretions that lead to chronic pulmonary disease, pancreatic insufficiency and intestinal obstructions, together with a

host of lesser but potentially lethal problems, such as an excessive loss of electrolytes in hot environments. In the past, afflicted children often died as infants. Although surviving into their twenties and thirties with current treatments, CF patients are plagued with recurrent infections and require daily arduous routines to clear air passageways.

5   In CF, mutations in the gene coding for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein result in defective Cl^- transport. The defect in the CFTR is also linked to hyperabsorption of Na^+ through the epithelial sodium channel (ENaC) (Boucher et al., 1986; Greger, 2000; Knowles et al., 1986; Mall et al., 1999) which is believed to account for an elevated basal short-circuit current (I_{sc}) in CF mucosal
10   epithelia and further to exacerbate the defect. This combination of ion transport abnormalities results in a reduced capacity to control airway surface liquid volume and reduced mucocilliary clearance, contributing to the pathophysiological conditions presenting in CF airways (Matsui et al., 2000; Matsui et al., 1998). The effort to correct the defective ion transport associated with CF has focused on the mechanisms modulating
15   ENaC, CFTR, and alternate Cl^- channel function. There are compelling arguments for pursuing artificial activation of alternate Cl^- channels to counteract CF pathophysiology. Mucosal epithelia express Cl^- channels other than the CFTR such as the outwardly rectifying chloride channel (ORCC), calcium activated Cl^- channels (CLCA) and volume regulated Cl^- channels. All are potential targets for CF treatment. In fact, the ORCC may
20   also be controlled by the CFTR and therefore be dysfunctional in CF (Clarke et al., 1994; Egan et al., 1992; Gabriel et al., 1993; Schwiebert et al., 1995). In contrast, Ca^{2+}-dependent Cl^- channels are reportedly more abundant in CF tissue (Grubb et al., 1994). A number of studies indicate that phenotypes with increased activity of alternate Cl^- channels such as the Ca^{2+} dependent Cl^- channels correlate with milder clinical
25   manifestations, (Clarke et al., 1994; Leung et al., 1995; Pilewski and Frizzell, 1999; Rozmahel et al., 1996; Veeze et al., 1994). Stimulation of apical Cl^- secretion through the CFTR and Ca^{2+} activated Cl^- channels has recently been found to be closely associated with ENaC function and sodium absorption in mucosal epithelia. (Devor and Pilewski, 1999; Inglis et al., 1999; Mall et al., 1999; Ramminger et al., 1999; Wang and Chan,
30   2000). Thus, it has been hypothesized that alternate Cl^- channels such as the Ca^{2+}-activated Cl^- channel and the ClC-x family may compensate for defects in CFTR function and could be utilized in a therapeutic strategy. This has lead to efforts to probe the usefulness of agents that elevate intracellular Ca^{2+}, such as purinergic agonists, in the

treatment of CF (Bennett et al., 1996). Currently two compounds are in development because they elevate intracellular calcium and thereby modulate Cl^- secretion, INS365 - a PY2Y receptor agonist, and duramycin - an antibiotic that triggers an increase in intracellular calcium levels.

5   However, an increase in intracellular Ca^{2+} does not always lead to Cl^- secretion. It has been demonstrated that the intracellular signaling molecule, inositol 3,4,5,6 tetrakisphosphate ($Ins(3,4,5,6)P_4$) "uncouples" chloride secretion from the rise in intracellular calcium in mucosal epithelia (Vajanaphanich, et al. 1994). This regulatory role for $Ins(3,4,5,6)P_4$ has been confirmed by several investigators (Ho et al., 1997; Xie et

10   al., 1998, Ismailov, et al., 1996).

Despite the foregoing advances, a need exists for new and improved compounds and methods for regulating ion transport in epithelial cells, such as by the modulation of ENaC.

Summary of the Invention

15   It has now been discovered that sodium ion absorption by epithelial cells can be modulated and inducible nitric oxide synthase (iNOS) can be inhibited *in vitro* or *in vivo* by certain camphanylidene and/or phenyl alkyl inositol polyphosphate derivatives. Accordingly, the present invention provides new compounds, compositions and methods of administering to a patient in need of such treatment a therapeutically effective amount

20   of a sodium uptake and/or inducible nitric oxide synthase (iNOS) inhibiting camphanylidene and/or phenyl alkyl inositol polyphosphate compound, or a stereoisomer, racemate, prodrug or a pharmaceutically acceptable salt thereof. In one aspect, the invention provides methods for inhibiting sodium ion absorption by epithelial cells and/or inhibiting inducible nitric oxide synthase (iNOS) in macrophages, comprising

25   administering to a patient in need of such treatment a therapeutically effective amount of a camphanylidene and/or phenyl alkyl inositol polyphosphate compound, such as 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4984), 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4996), 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol

30   3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4997) or a stereoisomer, racemate, or a pharmaceutically acceptable salt thereof. In another aspect of the invention, the invention provides methods for enhancing sodium ion absorption by epithelial cells, comprising administering to a patient in need of such treatment a

therapeutically effective amount of a sodium uptake enhancing camphanylidene and/or phenyl alkyl inositol polyphosphate compound, such as 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4984), 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4996), 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4997) or a stereoisomer, racemate, or a pharmaceutically acceptable salt thereof.

The methods, compounds and compositions of the invention may be employed alone, or in combination with other pharmacologically active agents in the treatment of disorders mediated by sodium ion absorption or of inducible nitric oxide synthase (iNOS), such as for treating pathological conditions related to cystic fibrosis, regulating fluid retention, regulating blood pressure in humans, treating inflammatory conditions, treating Alzheimer's disease, treating diabetes, treating pathological effects of ionizing radiation, and treating hyperproliferative disorders such as tumors, cancer, schleroderma, and hyperproliferative skin diseases such as psoriasis in human or animal subjects.

Brief Description of the Drawings

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 is a graph showing the effect of exposure to 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4996) and 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4984) on physiological parameters in CFHNE, I_{sc}, resistance and conductance, as described in Example 1. 10 µM of the test compound was added to the apical compartment of the Ussing chamber at the indicated time. Effects on I_{sc} in CFHNE, passage 2 are depicted. The monolayers were mounted in Ussing chambers and basal Isc, conductance and resistance measured. After a stable baseline was reached, amiloride was added to determine the amiloride-inhibitable-I_{sc}. Under these conditions, subsequent apical addition of the Ca^{2+}-mobilizing agent, ATP, allows Cl^- secretion to be examined in isolation. The graph shows I_{sc} in $\mu A/cm^2$.

FIGURE 2 is a graph showing the effect of exposure to two different concentrations of 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakis-

phosphate octakis (propionoxymethyl) ester (INO-4997; 1 and 10 μM) on physiological parameters (short circuit current) in CFHNE, as described in Example 1. Either 1 or 10 μM of the test compound or vehicle control was added to the apical compartment of the Ussing chamber at the indicated time.

FIGURE 3 is a graph showing amiloride inhibitable I_{sc} following addition of 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4997), as described in Example 1.

FIGURE 4 is a graph showing the prolonged effect of a 2 hour treatment with 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4997) measured 22 hours later in CFHNE cell monolayers, as described in Example 1.

FIGURE 5 is a graph showing the dose dependent inhibition of fluid absorption by 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4984) using the Blue Dextran Assay (Amil.: 100 micromolar amiloride), as described in Example 2.

FIGURE 6 is a graph showing the dose dependent inhibition of fluid absorption by 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4997) using the Blue Dextran Assay (Amil.: 100 micromolar amiloride), as described in Example 2.

FIGURE 7 is a graph showing the dose dependent inhibition of fluid absorption by 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propion-oxymethyl) ester (INO-4996) using the Blue Dextran Assay (Amil.: 100 micromolar amiloride), as described in Example 2.

FIGURE 8 is a graph showing the dose response of LPS in the inhibition of iNOS, as described in Example 3.

FIGURE 9 is a graph showing the dose response of dexamethasone (Dex) in the inhibition of iNOS, as described in Example 3.

FIGURE 10 is a graph showing the dose response of 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4997) in the inhibition of iNOS, as described in Example 3.

FIGURE 11 is a graph showing the dose response of 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4996) in the inhibition of iNOS, as described in Example 3.

FIGURE 12 is a graph showing the dose response of 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4984) in the inhibition of iNOS, as described in Example 3.

FIGURE 13 is a diagram showing the interrelationship of inositol signaling pathways with radiation exposure pathways regulating apoptosis and DNA repair, as described in Example 3.

### Detailed Description of the Preferred Embodiment

In accordance with the present invention, compounds, compositions and methods are provided for the regulation of sodium ion absorption by epithelial cells and/or inducible nitric oxide synthase (iNOS) by macrophages, either *in vitro* or *in vivo*. In one aspect, the present invention provides new camphanylidene and phenyl alkyl inositol polyphosphate derivative compounds that modulate the absorption of sodium ions in epithelial cells and the production of iNOS in macrophages. The invention also provides for pharmaceutical compositions containing the compounds and for the use of the compounds and compositions, alone or in combination with other pharmaceutically active agents. The invention additionally provides methods for inhibiting sodium ion absorption by cells and/or inducible nitric oxide synthase (iNOS) by macrophages, comprising administering to a patient in need of such treatment a therapeutically effective amount of a camphanylidene and/or phenyl alkyl inositol polyphosphate compound, or a stereoisomer, racemate, prodrug or a pharmaceutically acceptable salt thereof. In one aspect of the invention, the invention provides methods for inhibiting sodium ion absorption by epithelial cells and/or inducible nitric oxide synthase (iNOS) in macrophages, comprising administering to a patient in need of such treatment a therapeutically effective amount of a camphanylidene and/or phenyl alkyl inositol polyphosphate compound, or a stereoisomer, racemate, prodrug or a pharmaceutically acceptable salt thereof.

The sodium uptake inhibiting activity of the camphanylidene and/or phenyl alkyl inositol polyphosphate compounds of the invention may be determined by the cystic fibrosis human nasal epithelial (CFHNE) cell assay, as described in detail in Example 1, i.e., by mounting monolayers of human CF nasal epithelial cells in Ussing chambers, and then monitoring short-circuit current (I_{sc}) and resistance after contact with a test inositol polyphosphate compound. Sodium uptake inhibiting inositol polyphosphate compounds generally exhibit reduced I_{sc}, and increased resistance relative to controls. Sodium uptake
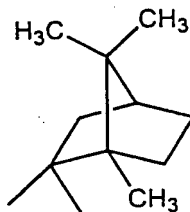
enhancing inositol polyphosphate compounds generally exhibit increased I_{sc}, and decreased resistance relative to controls.

The presently particularly preferred sodium uptake inhibiting camphanylidene and/or phenyl alkyl inositol polyphosphate compounds useful in the practice of the invention include any camphanylidene and/or phenyl alkyl inositol polyphosphate compounds that inhibit I_{sc} and increase resistance relative to controls as determined by the CFHNE cell assay.

The camphanylidene inositol polyphosphate compounds of the invention will generally be compounds of the formula:
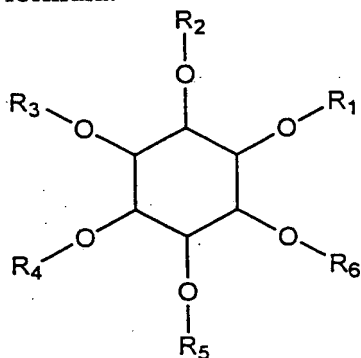
wherein two adjacent substituents of R_1-R_6 are taken together to form a camphanylidene group of the formula:

and the remainder of R_1-R_6 are independently selected from hydrogen, $-PO(O-R_7)_2$, $-C_1-C_{20}$ straight or branched chain alkyl, $-C_2-C_{20}$ straight or branched chain alkenyl or alkynyl, $-OC(O)C_1-C_{20}$ straight or branched chain alkyl and $-OC_1-C_{20}$ straight or branched chain alkyl, and $-OC_2-C_{20}$ straight or branched chain alkenyl or alkynyl;

each R_7 is independently selected from a group consisting of hydrogen and $-C(R_8)(R_8)OC(O)C_1-C_4$ straight or branched chain alkyl; and
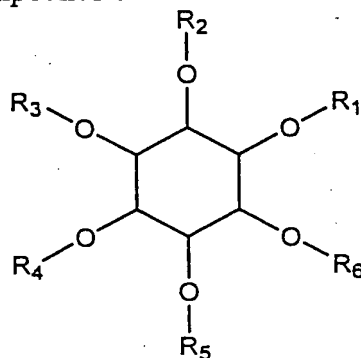
each R_8 is independently selected from a group consisting of hydrogen and $-C_1-C_{12}$ alkyl, both R_8 taken as a 5- or 6-membered ring, phenyl, and benzyl, said R_8,

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except hydrogen, being unsubstituted or substituted with one or more halogen, -OH, C_1-C_6 alkyl, NO_2, -OC_1-C_6 alkyl, and $OC(O)C_1$-C_6 alkyl groups;

and the stereoisomers, racemates and pharmaceutically acceptable salts thereof.

Presently preferred and representative camphanylidene inositol polyphosphate compounds for use in the practice of the invention include, for example, 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester, and 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester.

The phenylalkyl inositol polyphosphate compounds useful in the practice of the invention will generally be compounds of the formula:

wherein at least one of R_1-R_6 is a phenylalkyl group of the formula:
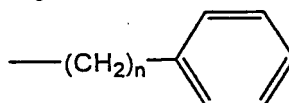
wherein n is 1-10; and the remainder of R_1-R_6 are independently selected from hydrogen, -$PO(O$-$R_7)_2$, -C_1-C_{20} straight or branched chain alkyl, -C_2-C_{20} straight or branched chain alkenyl or alkynyl, -$OC(O)C_1$-C_{20} straight or branched chain alkyl and -OC_1-C_{20} straight or branched chain alkyl, and -OC_2-C_{20} straight or branched chain alkenyl or alkynyl;

each R_7 is independently selected from a group consisting of hydrogen and -$C(R_8)(R_8)OC(O)C_1$-C_4 straight or branched chain alkyl; and

each R_8 is independently selected from a group consisting of hydrogen and -C_1-C_{12} alkyl, both R_8 taken as a 5- or 6-membered ring, phenyl, and benzyl, said R_8, except hydrogen, being unsubstituted or substituted with one or more halogen, -OH, C_1-C_6 alkyl, NO_2, -OC_1-C_6 alkyl, and $OC(O)C_1$-C_6 alkyl groups;

and the stereoisomers, racemates and pharmaceutically acceptable salts thereof.

A presently preferred and representative phenylalkyl inositol polyphosphate compound for use in the practice of the invention is 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester.

In presently particularly preferred embodiments, the camphanylidene and/or phenyl alkyl inositol polyphosphate compounds of the invention are designed to be delivered intracellularly as prodrugs, such as by concealing the negatively charged phosphate groups with bioactivatable esters, such as acetoxymethylesters (AM-esters), propionoxymethylesters (PM-esters) or pivaloyloxymethyl esters, and the hydroxy groups with alkyl groups, such as butyrates, where necessary. These masking groups have previously been shown to permit passive diffusion of other inositol polyphosphate compounds across the plasma membrane to the interior of the cell where esterases cleave them and liberate the biologically active inositol polyphosphate inside the cell. (See M. Vajanaphanich et al., *Nature* <u>371</u>:711 (1994); Rudolf, M. T. et al., "2-Deoxy derivative is a partial agonist of the intracellular messenger inositol 3,4,5,6-tetrakisphosphate in the epithelial cell line T84" *J Med Chem* 41:3635-44 (1998)).

Compounds of the present invention can be readily synthesized using the methods described herein, or other methods, which are well known in the art. See, for example, Jiang, T. et al., "Membrane-permeant Esters of Phosphatidylinositol 3,4,5-Trisphosphate," *J. Bio. Chem.* 273(18):11017-11024 (1998) and Bruzik, K.S. et al., "Efficient and Systematic Syntheses of Enantiomerically Pure and Regiospecifically Protected *myo*-Inositols," *J. Am. Chem. Soc.* 114:6361-6374 (1992). More specifically, the camphanylidene compounds may be synthesized by following the following reaction scheme 1:
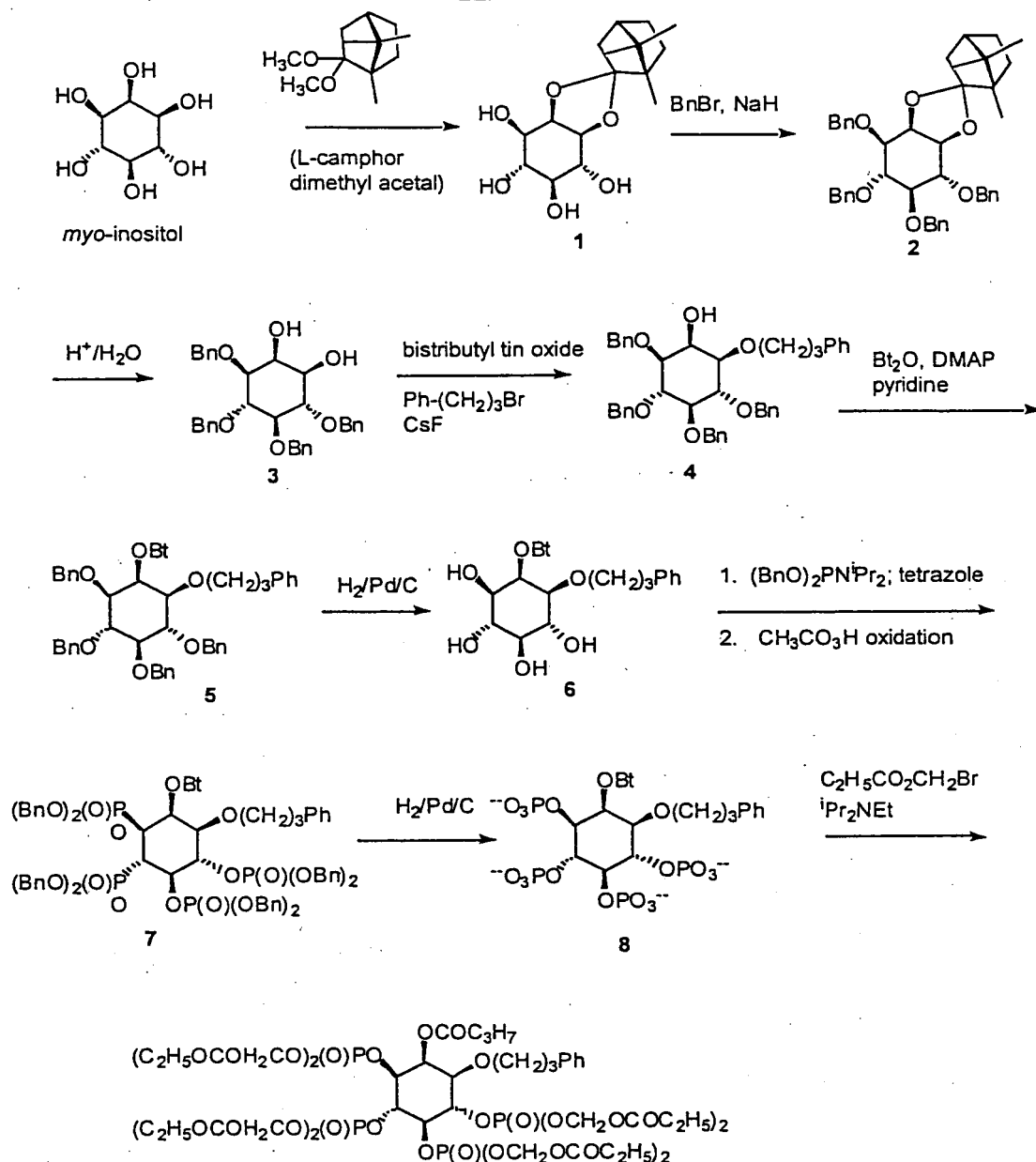
Scheme 1
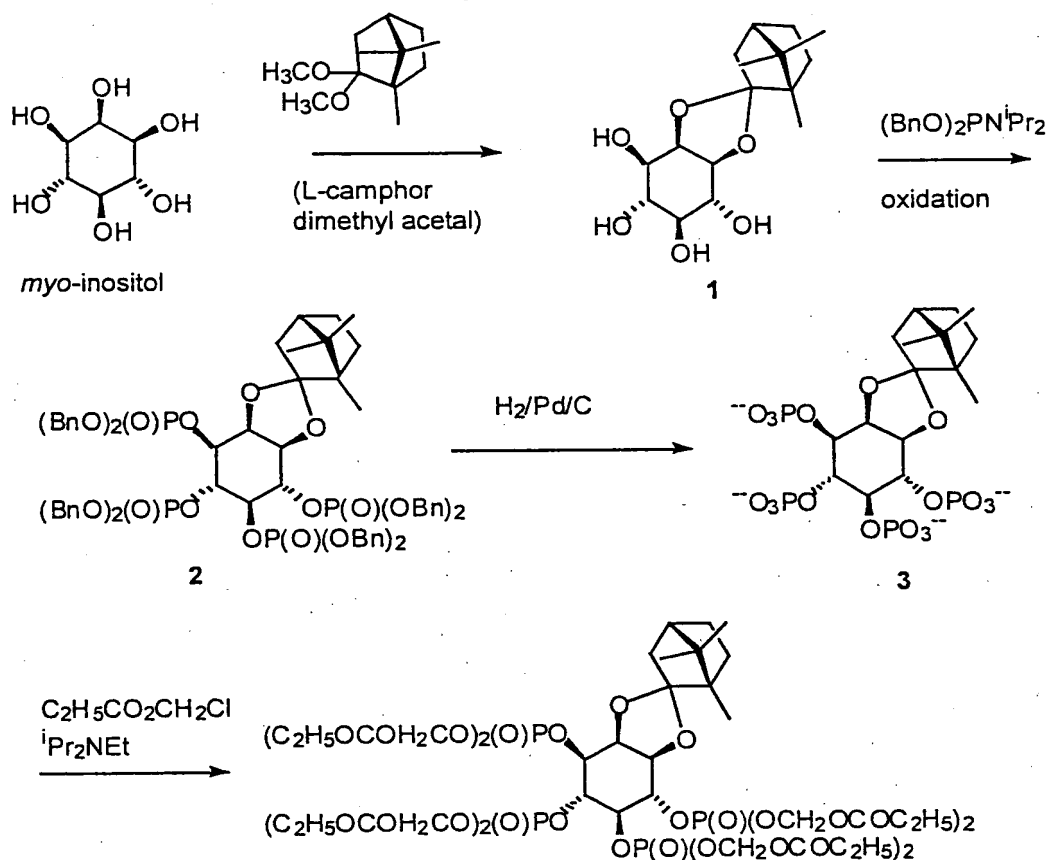
Referring to reaction scheme 1, the compound 1,2-camphanylidene-*myo*-inositol

3,4,5.6-tetrakisphosphate-octakis(proprionoxymethyl) ester is synthesized as follows.

5   Transacetalization of *myo*-inositol by L-camphor dimethyl acetal, prepared in one step

from commercially available L-camphor, is carried out in the presence of sulfuric acid,

and afforded 1,2-ketal **1** by crystallization from methanol in about 50-60% yield. (Bruzik,

K.S. and Tsai, M., *J. Am Chem. Soc.* 114:6361-6374 (1992)). Using standard inositol

phosophorylation conditions (as described in the US patents 5,977,078 and 5,880,099),

10   the ketal tetrol **1** is phosphorylated by treatment with the phosphoramidite $(BnO)_2PN^iPr_2$

and tetrazole in acetonitrile, with subsequent oxidation of the phosphite intermediate with

peracetic acid at -40°C to yield the tetrakis(dibenzyl)phosphate **2**, purified by flash

chromatography, with a yield of about 40%. The phosphate groups are deprotected using

hydrogen gas over palladium catalyst, a standard method for hydrogenolysis of benzyl

15   phosphates (also described in the above cited patents) providing **3** without the need for

additional purification.   The   tetraphosphate   **3**   is   then   alkalized   using

propionoxymethylene bromide and diisopropylethylamine, resulting in 1,2-camphanylidene-*myo*-inositol 3,4,5.6-tetrakisphosphate octakis(proprionoxymethyl) ester (INO-4996).

The phenylalkyl compounds of the invention may be synthesized by following the

5   following reaction scheme 2:

Scheme 2a

Referring to reaction scheme 2, the compound 2-O-butyryl-1-O-(3-phenylpropyl)-myo-inositol-3,4,5,6-tetrakisphosphate octakis(proprionoxymethyl) ester is synthesized as follows. Transacetalization of myo-inositol by L-camphor dimethyl acetal, prepared in one step from commercially available L-camphor, is carried out in the presence of sulfuric acid, and affords 1,2-ketal **1** by crystallization from methanol in about 50-60% yield. (Bruzik, K.S. and Tsai, M., *J. Am Chem. Soc.* 114:6361-6374 (1992)). The ketal **1** is alkylated with benzyl bromide/sodium hydride in THF to produce the fully protected

compound 2, which is subjected to acidic deacetalization in methanol to produce the tetrabenzyl inositol 3. The pure product is obtained by crystallization from hexane, ensuring high enantiomeric purity of this and the downstream products. The 1,2-diol 3 is converted into the dibutyl stannane with bistributyltin oxide, then alkylated with 3-phenylpropyl bromide/cesium fluoride to produce alcohol 4. The diasteromeric products are separated by column chromatography. The purified alcohol 4 is acylated with butyric anhydride /DMAP in pyridine to yield the fully protected inositol 5. Protected inositol 5 is subjected to hydrogenolysis with hydrogen over palladium on carbon catalyst at less than 50 psi. This is a standard method (described in the US patents 5,977,078 and 5,880,099) for benzyl group removal, and produces tetrol 6. Using standard inositol phosophorylation conditions (also described in the patents cited above), the tetrol 6 is phosphorylated by treatment with the phosphoramidite $(BnO)_2PN^iPr_2$ and tetrazole in acetonitrile. Subsequent oxidation of the phosphite intermediate with peracetic acid at -40°C yields the tetrakis(dibenzyl)phosphate 7. The phosphate groups are deprotected using hydrogen over palladium catalyst, producing tetraphosphate 8. This material is then alkylated using propionoxymethylene bromide and diisopropylethylamine, resulting in 2-O-butyryl-1-O-(3-phenylpropyl)-myo-inositol-3,4,5,6-tetrakisphosphate octakis(proprionoxymethyl) ester (INO-4997).

Compounds of the invention may be tested *in vivo* to demonstrate efficacy of the compounds in remediating the symptoms of cystic fibrosis and/or cardiovascular disease. For example, indices measured *in vivo* that demonstrate the efficacy of compounds include measurement of the effects of the compounds in animals such as mice and human beings in nasal potential difference (NPD) as described in Knowles, M. R., Paradiso, A. M., and Boucher, R. C. (1995). In vivo nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. *Hum Gene Ther 6*, 445-55; mucociliary clearance of [^{99m}Tc] iron oxide particles as described in Bennett, W. D., Olivier, K. N., Zeman, K. L., Hohneker, K. W., Boucher, R. C., and Knowles, M. R. (1996). Effect of uridine 5'-triphosphate plus amiloride on mucociliary clearance in adult cystic fibrosis. Am J Respir Crit Care Med *153*, 1796-801 and Olivier, K. N., Bennett, W. D., Hohneker, K. W., Zeman, K. L., Edwards, L. J., Boucher, R. C., and Knowles, M. R. (1996). Acute safety and effects on mucociliary clearance of aerosolized uridine 5'-triphosphate +/- amiloride in normal human adults. Am J Respir Crit Care Med *154*, 217-23; forced expiratory volume 1 (FEV1); measurement of the production of inflammatory

mediators and cytokines such as leukotrienes, interleukins, complement factors and platelet activating factor as described in Coffer, P. J., Geijsen, N., M'Rabet, L., Schweizer, R. C., Maikoe, T., Raaijmakers, J. A., Lammers, J. W., and Koenderman, L. (1998). Comparison of the roles of mitogen-activated protein kinase and

5   phosphatidylinositol 3-kinase signal transduction in neutrophil effector function. Biochem J *329*, 121-30, and Gibbs, B. F., Schmutzler, W., Vollrath, I. B., Brosthardt, P., Braam, U., Wolff, H. H., and Zwadlo-Klarwasser, G. (1999). Ambroxol inhibits the release of histamine, leukotrienes and cytokines from human leukocytes and mast cells. Inflamm Res *48*, 86-93. Such tests as well as a complete blood count show whether

10   secondary infections and ensuing inflammatory responses are ameliorated by treatment. Blood pressure can also be monitored. For determining whether extrapulmonary manifestations are corrected, fecal fat can be evaluated.

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids. These salts include but are not limited to the following:

15   acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, nicotinate,

20   2-napthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylproionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, p-toluenesulfonate and undecanoate.

Examples of acids which may be employed to form pharmaceutically acceptable acid addition salts include such inorganic acids as hydrochloric acid, sulphuric acid and phosphoric acid and such organic acids as oxalic acid, maleic acid, succinic acid and

25   citric acid. Basic addition salts can be prepared *in situ* during the final isolation and purification of the compounds, or separately by reacting carboxylic acid moieties with a suitable base such as the hydroxide, carbonate or bicarbonate of a pharmaceutical acceptable metal cation or with ammonia, or an organic primary, secondary or tertiary amine. Pharmaceutical acceptable salts include, but are not limited to, cations based on

30   the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, aluminum salts and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine,

trimethylamine, triethylamine, ethylamine, and the like. Other representative organic amines useful for the formation of base addition salts include diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

The compounds of the invention are useful *in vitro* for inhibiting sodium ion absorption in a cell or tissue, and *in vivo* in human and animal hosts for the regulation of the sodium channel, ENaC. The compounds may be used alone or in compositions together with a pharmaceutically acceptable carrier.

Thus, in one aspect, the present invention provides methods of treatment of cystic fibrosis in a subject in need of such treatment by administering an inositol polyphosphate as given above to the subject in an amount effective to modulate epithelial sodium ion absorption. In another aspect, the present invention provides methods of treating chronic bronchitis in a subject in need of such treatment by administering an inositol polyphosphate as given above to the subject in an amount effective to modulate epithelial sodium ion absorption. In another aspect, the present invention provides methods of treating asthma in a subject in need of such treatment by administering an inositol polyphosphate analog as given above to the subject in an amount effective to modulate epithelial sodium ion absorption. In another aspect, the present invention provides methods of combating chronic obstructive pulmonary disorder by administering an inositol polyphosphate analog as given above to said subject in an amount effective to modulate epithelial sodium ion absorption. In another aspect, the present invention provides methods of regulating fluid retention by administering an inositol polyphosphate analog as given above to the subject in an amount effective to modulate epithelial sodium ion absorption. In another aspect, the present invention provides methods of regulating blood pressure by administering an inositol polyphosphate analog as given above to said subject in an amount effective to modulate epithelial sodium ion absorption. In yet other aspects, the present invention provides methods of use of an the active compounds as disclosed herein for the manufacture of a medicament for the prophylactic or therapeutic treatment of cystic fibrosis in a subject in need of such treatment. In yet other aspects, the present invention provides methods of use of the active compounds as disclosed herein for the manufacture of a medicament for the prophylactic or therapeutic treatment of chronic bronchitis in a subject in need of such treatment. In yet other aspects, the present invention provides methods of use of an the active compounds as disclosed herein

for the manufacture of a medicament for the prophylactic or therapeutic treatment of asthma in a subject in need of such treatment.

When administered to a patient, *e.g.*, a mammal for veterinary use or to a human for clinical use, the inositol derivatives are preferably administered in isolated form. By "isolated" is meant that prior to formulation in a composition, the inositol derivatives are separated from other components of either (a) a natural source such as a plant or cell culture, or (b) a synthetic organic chemical reaction mixture. Preferably, via conventional techniques, the inositol derivatives are purified.

When administered to a patient, *e.g.*, a mammal for veterinary use or to a human for clinical use, or when made to contact a cell or tissue, the inositol derivatives can be used alone or in combination with any physiologically acceptable carrier or vehicle suitable for enteral or parenteral delivery. Where used for enteral, parenteral, topical, otic, ophthalmologic, intranasal, oral, sublingual, intramuscular, intravenous, subcutaneous, intravaginal, transdermal, or rectal administration, the physiologically acceptable carrier or vehicle should be sterile and suitable for *in vivo* use in a human, or for use in a veterinary clinical situation.

In addition, the inositol derivatives can be administered to patients or contacted with a cell or tissue in liposome formulations, which facilitate their passage through cell membranes. Accordingly, the relative impermeability of cell membranes to relatively polar inositol derivatives can be overcome by their encapsulation in liposomal formulations. The characteristics of liposomes can be manipulated by methods known to those of ordinary skill in the art, such that size, membrane fluidity, tissue targeting, and compound release kinetics are adapted to the particular condition (Georgiadis, *NIPS* 4:146 (1989)). Liposomes of various sizes and compositions that encapsulate the inositol derivatives for delivery can be achieved by methods known to those skilled in the art (See, for example, Hope et al., *Biochem. Biophys. Acta* 812:55 (1985); Hernandez, et al., *J. Microencapsul.* 4:315 (1987); Singh, et al., *Cancer Lett.* 84:15 (1994); and Dipali, et al., *J. Pharm. Pharmacol.* 48:1112 (1996)).

The inositol derivatives can be used in the form of a pharmaceutical preparation, for example, in solid, semisolid or liquid form, that contains at least one of the inositol derivatives of the present invention as a bioactive component, alone or in combination with an anti-inflammatory compound, in admixture with a carrier, vehicle or an excipient suitable for enteral or parental administration. Such anti-inflammatory compounds useful

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in this regard include, but are not limited to, non-steroidal anti-inflammatory drugs such as salicylic acid, acetylsalicylic acid, methyl salicylate, diflunisal, salsalate, olsalazine, sulfasalazine, acetaminophen, indomethacin, sulindac, etodolac, mefenamic acid, meclofenamate sodium, tolmetin, ketorolac, dichlofenac, ibuprofen, naproxen, naproxen

5   sodium, fenoprofen, ketoprofen, flurbinprofen, oxaprozin, piroxicam, meloxicam, ampiroxicam, droxicam, pivoxicam, tenoxicam, nabumetome, phenylbutazone, oxyphenbutazone, antipyrine, aminopyrine, apazone and nimesulide; leukotriene antagonists including, but not limited to, zileuton, aurothioglucose, gold sodium thiomalate and auranofin; and other anti-inflammatory agents including, but not limited

10   to, colchicine, allopurinol, probenecid, sulfinpyrazone and benzbromarone.

In addition, the inositol derivatives of the present invention may be compounded, for example with a pharmaceutically acceptable carrier or vehicle for solid compositions such as tablets, pellets or capsules; capsules containing liquids; suppositories; solutions; emulsions; aerosols; sprays; suspensions or any other form suitable for use. Suitable

15   carriers and vehicles include, for example, sterile water, sterile physiological saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. The inositol derivatives are present in the compositions in a therapeutically effective amount, *i.e.*, an amount sufficient to restore normal mucosal secretions.

20   The compositions of this invention may be administered by a variety of methods including orally, sublingually, intranasally, intramuscularly, intravenously, subcutaneously, intravaginally, transdermally, rectally, by inhalation, or as a mouthwash in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration may also

25   involve the use of transdermal administration such as transdermal patches or ionophoresis devices. The preferred mode of administration is left to the discretion of the practitioner, and will depend in-part upon the desired site of action.

For example, when cystic fibrosis, chronic bronchitis or asthma affects the function of the lungs, the inositol derivatives can be administered as an atomized aerosol,

30   via a nebulizer, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant; alternatively, the inositol derivatives can be administered intravenously directly. Thus, the active compounds disclosed herein may be administered to the lungs of a patient by any suitable means, but are preferably administered by generating an aerosol comprised

of respirable particles, the respirable particles comprised of the active compound, which particles the subject inhales. The respirable particles may be liquid or solid. The particles may optionally contain other therapeutic ingredients such as a sodium channel blocker as noted above, with the sodium channel blocker included in an amount effective to inhibit the reabsorption of water from airway mucous secretions. The particles may optionally contain other therapeutic ingredients such as antibiotics as described in Patents 5,512,269 and 5,716,931 or Uridine Triphosphate Analogs as described in Patent 5,292,498, nitric oxide inhibitors as described in Patent 5,859,058, dinucleotides as described in Patent 5,935,555, or organic acids as described in Patent 5,908,611. Particles comprised of active compound for practicing the present invention should include particles of respirable size: that is, particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about 0.5 to 10 microns in size (more particularly, less than about 5 microns in size) are respirable. Particles of non-respirable size which are included in the aerosol tend to deposit in the throat and be swallowed, and the quantity of non-respirable particles in the aerosol is preferably minimized. For nasal administration, a particle size in the range of 10-500 μm is preferred to ensure retention in the nasal cavity.

Liquid pharmaceutical compositions of active compound for producing an aerosol can be prepared by combining the active compound with a suitable vehicle, such as sterile pyrogen free water. Other therapeutic compounds, such as a sodium channel blocker, may optionally be included. Solid particulate compositions containing respirable dry particles of micronized active compound may be prepared by grinding dry active compound with a mortar and pestle, and then passing the micronized composition through a 400 mesh screen to break up or separate out large agglomerates. A solid particulate composition comprised of the active compound may optionally contain a dispersant that serves to facilitate the formation of an aerosol. A suitable dispersant is lactose, which may be blended with the active compound in any suitable ratio (e.g., a 1 to 1 ratio by weight). Again, other therapeutic compounds may also be included.

The dosage of active compound for prophylaxis or treatment of lung disease will vary depending on the condition being treated and the state of the subject, but generally may be an amount sufficient to achieve dissolved concentrations of active compound on the airway surfaces of the subject of from about 10^{-9} to 10^{-3} Moles/liter, and more

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preferably from 10^{-7} to 10^{-5} Moles/liter. Depending on the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose administrations. Preferably, the daily dose is a single unit dose, which is preferably administered from 1 to 3 times a week. Treatments may continue

5   week to week on a chronic basis as necessary (i.e., the active agent can be administered chronically). Administration of the active compounds may be carried out therapeutically (i.e., as a rescue treatment) or prophylactically, but preferably the compounds are administered prophylactically, either before substantial lung blockage due to retained mucus secretions has occurred, or at a time when such retained secretions have been at

10   least in part removed, as discussed above.

Aerosols of liquid particles comprising the active compound may be produced by any suitable means, such as with a nebulizer. See, e.g., U.S. Pat. No. 4,501,729. Nebulizers are commercially available devices that transform solutions or suspensions of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a

15   compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier, the active ingredient comprising up to 40% w/w of the formulation, but preferably less than 20% w/w. the carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of,

20   for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl hydroxybenzoate, antioxidants, flavoring agents, volatile oils, buffering agents and surfactants. Aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate

25   medicaments to a subject produce particles that are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an

30   insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder (e.g., a metered dose thereof effective to carry out the treatments described herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in situ and the powder delivered by air drawn through the

device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquefied propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 150 µl, to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation may additionally contain one or more co-solvents, for example, ethanol, surfactants, such as oleic acid or sorbitan trioleate, antioxidants and suitable flavoring agents. The aerosol, whether formed from solid or liquid particles, may be produced by the aerosol generator at a rate of from about 10 to 150 liters per minute, more preferably from about 30 to 150 liters per minute, and most preferably about 60 liters per minute. Aerosols containing greater amounts of medicament may be administered more rapidly.

Where the condition of the subject to be treated affects the gastrointestinal tract, the inositol derivatives can be administered rectally via enema or suppository, or orally in the form of a tablet or capsule formulated to prevent dissolution prior to entry into the afflicted portion of the gastrointestinal tract; when the cystic fibrosis affects vaginal secretions, the inositol derivatives can be administered intravaginally, in the form of a douche.

Compositions for oral delivery may be in the form of tablets, pills, troches, lozenges, aqueous or oily suspensions, granules or powders, emulsions, capsules, syrups or elixirs. Orally administered compositions may contain one or more agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, compositions in tablet form may be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also

suitable for orally administered compositions. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time delay material such as glycerol monostearate or glycerol stearate may also be used. Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleagenous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1/3-propanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols, which are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Aqueous suspensions containing the inositol derivatives may also contain one or more preservatives, such as, for example, ethyl or n-propyl-p-hydroxy-benzoate, one or more coloring agents, flavoring agents or sweetening agents.

Because the preferred inositol derivatives are in the form of tetrakisphosphate, heptakis or octakis(acetoxymethyl or ethyl)esters, and because the inositol derivatives can contain $-C_1-C_{20}$ straight or branched chain alkyl, $-OC(O)C_1-C_{20}$ straight or branched chain alkyl or $-OC_1-C_{20}$ straight or branched chain alkyl groups, the inositol derivatives possess enhanced lipophilic properties which allow for passive diffusion across plasma

membranes. This design permits the inositol derivatives to more easily penetrate cell membranes and travel to sites more easily and quickly.

The compounds of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.W. (1976), p.33 *et seq*.

Without being bound by any particular theory, it is believed that the ester protected inositol derivatives of the invention function as "prodrugs" of a metabolized form of the inositol derivatives that are the actual pharmacological agent responsible for the modulation of sodium ion absorption. Such prodrugs, by virtue of their being more lipophilic than the actual pharmacological agents themselves, can more easily penetrate plasma membranes. Once within a secretory cell, the prodrugs are converted, generally enzymatically, to the active pharmacological agent. In addition, because *in vivo* conversion of a prodrug to its active pharmacological form generally occurs over a period of time, rather than instantaneously, the use of prodrugs offers the patient or subject the benefit of a sustained release of the pharmacological agent, generally resulting in a longer duration of action.

In addition, without being bound by any particular theory, it is believed that the inositol derivatives, by virtue of the fact that they comprise phosphate ester groups, are able to accumulate within "depots," *i.e.*, fatty domains of the brain, in particular, within cell membranes. Within in such depots, the inositol derivatives act to inhibit tissue damage caused by inflammation.

In a further embodiment, the present invention contemplates the use of an inositol derivative when delivered at a dose of about 0.001 mg/kg to about 100 mg/kg body weight, preferably from about 0.01 to about 10 mg/kg body weight. The inositol derivatives can be delivered up to several times per day, as needed. Treatment can be

continued, indefinitely to normalize mucosal hydration or sodium absorption or reduce excessive mucosal viscosity.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the

5   particular mode of administration.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the

10   severity of the particular disease undergoing therapy.

While the compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more other agents used in the treatment of the symptoms of cystic fibrosis, chronic bronchitis, asthma, inflammation and the like. For alleviating mucosal viscosity resulting from cystic

15   fibrosis, a composition of the present invention may be administered that comprises an inositol derivative of the invention together with an agent useful for the treatment of inflammation-accompanying condition. For instance, for the treatment of cystic fibrosis, such an agent can be mucolytics (e.g., Pulmozyme® and Mucomyst®), purinergic receptor agonists such as uridine triphosphate (UTP), agents that suppress the cystic fibrosis

20   transmembrane regulator (CFTR) premature stop mutation such as gentamycin, agents correcting the Delta F508 processing defect also known as "protein assist therapies" such as CPX™ (SciClone), Phenylbutyrate (Ucyclyd Pharma), INS365 (Insprie Pharmaceuticals), and genestein, and/or agents for the treatment of the accompanying infection such as tobramycin or aerosolized tobramycin (Tobi™), meropenem, RSV

25   vaccine, IB605, Pa1806, anti-inflammatory agents such as DHA, rHEI, DMP777, IL10 (Tenovil) and/or agents triggering alternate chloride channels such as antibiotics such as Duramycin (Moli901 - Molichem Medicines), or omeprazole, and/or purinergic agonists such as nucleotide or dinucleotide analogs, or agents affecting sodium transport such as amiloride, and/or agents affecting pH such as organic acids.

30   For the treatment of asthma, such agents can be corticosteroids – such as fluticasone propionate (Flovent®, Flovent Rotadisk®), budesonide (Pulmocort Turbuhaler®), flunisolide (Aerobid®), triamcinolone acetonide (Azmacort®), beclomethasone MDI (Beclovent®), antileukotrienes such as Zafirlukast (Accolate®,

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Zeneca®), Zileuton (Zyflo®), Montelukast or other therapies such as methotrexate, troleandomycin, gold, cyclosporine, 5'-lipoxygenase inhibitors, bronchodilators, or immunotherapeutic agents.

5   CPX is a caffeine-like compound being investigated by SciClone. In laboratory studies it appears to increase chloride secretion in CF tissues that have the delta F508 mutation, but not in tissues with other mutations or normal epithelial cells. It is unknown whether it would be effective in actual patients. Even if so, it would not benefit the 30% of CF sufferers who have other mutations.

        Phenylbutyrate is a compound developed by Ucyclyd Pharma that targets the
10   protein generated by the delta F508 mutation. The Cystic Fibrosis Foundation is currently sponsoring a Phase I clinical trial of the drug at the Johns Hopkins University. However, because high concentrations are necessary to be effective and the compound has an unappealing odor, other active analogs are currently being sought.

        Duramycin is being developed by Molichem Medicines and forms pores in
15   membranes allowing the passage of ions. However, it is difficult to regulate the concentration of the compound in the membrane and the efficacy of the compound.

        Purinergic (P2Y2) receptor agonists such as adenosine triphosphate (ATP) and uridine triphosphate (UTP) stimulate calcium-dependent chloride channels (not CFTR channels). They are currently being investigated by researchers at the University of
20   North Carolina (under the auspices of Inspire Pharmaceuticals, Inc.) and independently at Johns Hopkins University. Early trials indicate that this strategy could be useful in the treatment of cystic fibrosis and other chronic obstructive pulmonary disorders. However, the effectiveness of this approach may be limited by inflammation-related inhibitory signals.

25   The compounds of the invention may also be administered in combination with one or more sodium channel blockers. Sodium channel blockers which may be used in the present invention are typically pyrazine diuretics such as amiloride, as described in U.S. Pat. No. 4,501,729. The term "amiloride" as used herein includes the pharmaceutically acceptable salts thereof, such as (but not limited to) amiloride
30   hydrochloride, as well as the free base of amiloride. The quantity of amiloride included may be an amount sufficient to achieve dissolved concentrations of amiloride on the airway surfaces of the subject of from about 10^{-7} to about 10^{-3} Moles/liter, and more preferably from about 10^{-6} to about 10^{-4} Moles/liter.

The methods of the present invention may also further comprise the step of removing retained mucus secretions from the lungs of the subject prior to the step of administering the active agent. This facilitates application of the active agent to the respiratory epithelia during the administering step. Such removal of retained mucus

5   secretions can be carried out by any suitable means, including postural drainage, antibiotic administration (e.g., intravenous or inhalation administration of cephalosporin or aminoglycoside antibiotics such as Tobramycin), and/or inhalation administration of DNase. In addition, the present invention may be carried out on patients such as children prior to decline of respiratory function (e.g., patients essentially free of lung blockage due

10   to retained mucus secretions). Such patients can be genetically predisposed to becoming afflicted with lung disease (e.g., cystic fibrosis) as hereinbefore described.

Alternatively, the compositions comprising an inositol derivative can be administered in combination with, prior to, concurrent with or subsequent to the administration of another agent useful for the treatment of cystic fibrosis accompanying

15   condition, as described above.

In addition, the inositol derivatives can be used for research purposes; for example, to investigate the mechanism and activity of other agents thought to be useful for regulating mucosal hydration.

The foregoing may be better understood by reference to the following examples,

20   which are provided for illustration and are not intended to limit the scope of the inventive concepts.

<div align="center">EXAMPLE 1.</div>

<div align="center">Effect of Test Compounds on Basal Spontaneous I_{sc} In</div>

<div align="center">Cystic Fibrosis Human Nasal Epithelial Cell Ussing Chamber Assay</div>

25   Epithelia derived from individuals with CF are unique and display a hyperabsorptive phenotype due to defective cystic fibrosis transmembrane conductance regulator (CFTR) with concomitant loss of a Cl^- conduit and dysregulation of Na^+ absorption through the amiloride-sensitive Na^+ channel, ENaC (Stutts, M.J. et al., "CFTR as a cAMP-dependent regulator of sodium channels," *Science* 269:847-850 (1995); Stutts,

30   M.J. et al., "Cystic fibrosis transmembrane conductance regulator inverts protein kinase A-mediated regulation of epithelial sodium channel single channel kinetics," *J Biol Chem.* 272:14037-14040 (1997)). ENaC is the rate limiting step in the regulation of sodium absorption across mucosal epithelia and as such, is an essential effector in the

maintenance of airway surface liquid volume/depth (Knowles, M.R. et al., "Abnormal ion permeation through cystic fibrosis respiratory epithelium," *Science* 221:1067-70 (1983)). Excess fluid/volume absorption has been correlated with defects in ENaC regulation in CF and plays a primary role in the reduced mucociliary clearance found in CF airways

5   (Jiang, C. et al., "Altered fluid transport across airway epithelium in cystic fibrosis," *Science* 262:424-7 (1993); Sood, N. et al., "Increasing concentration of inhaled saline with or without amiloride: effect on mucociliary clearance in normal subjects," *Am J Respir Crit Care Med.* 167:158-63 (2003)). Amiloride, an extracellular blocker of ENaC, has been shown in clinical trials to temporarily increase mucociliary clearance (Knowles,

10   M.R. et al., "Mucus clearance as a primary innate defense mechanism for mammalian airways," *J Clin Invest* 109:571-7 (2002)) . However, the short duration of amiloride action, presumably due to the internalization of ENaC and the removal of effective concentrations of extracellular amiloride, limits this therapeutic strategy.

In contrast to extracellularly acting agents directed against the extracellular
15   domain of ion channel pores, membrane-permeant inositol polyphosphate analogs modulate ion channel activities from inside the cell. This effect is long-lasting because these compounds are very slowly metabolized by intracellular enzymes (Tomkiewicz, R.P. et al., "Amiloride inhalation therapy in cystic fibrosis. Influence on ion content, hydration, and rheology of sputum," *Am Rev Respir Dis* 148:1002-7 (1993)). Therefore,

20   they have the potential for prolonged activity in contrast to extracellularly active compounds that are rapidly eliminated from the airway surface liquid. We describe the effects of analogs of *myo*-inositol 3,4,5,6-tetrakisphosphate, INO-4997, INO-4996, and INO-4984, on two parameters predictive of airway surface liquid volume in CF airway epithelia, basal amiloride inhibitable short circuit current and fluid absorption rate.

25   ***CF Human Nasal Epithelial (CFHNE) Cell Isolation and Proliferation***: Surgically removed nasal polyps were obtained from volunteers in collaboration with Dr. Bonnie Ramsey at Children's Hospital, Seattle and Dr. Ludwig Allegra at the Northwest Nasal Sinus Center, and transported on ice in a sterile container containing a 1:1 mixture of Dulbecco's modification of minimum essential medium Eagle and Ham's F-12 nutrient

30   medium (DMEM/F-12)(Irvine Scientific, Santa Ana, CA) supplemented with 100 U/ml penicillin, 0.1mg/ml streptomycin, 10mM HEPES, and 2mM L-glutamine. The tissue samples were aseptically removed from the transport medium and washed (repeated 5X) by suspending in 40ml of Joklik's modification of minimum essential medium Eagle

(JMEM) at 4°C, and centrifuging at 500 RPM. The supernatant was aspirated and discarded. The tissue was then transferred to JMEM containing 200 U/ml penicillin, 0.2mg/ml streptomycin, 0.1mg/ml gentamicin sulfate (Clonetics, San Diego, CA), and 0.1μg/ml amphotericin-B (Clonetics), and 0.1% Protease (Sigma), washed an additional

5   2X, suspended in 15ml in a 10cm tissue culture dish, and incubated at 4°C for 24 hours. The tissue samples were then gently triturated, the connective tissue aseptically removed, and the remaining cell suspension centrifuged at 1000 RPM for 5min. The supernatant was aspirated and the pellet was resuspended in 10ml JMEM with 0.025% trypsin-EDTA and allowed to incubate for 5 min. After 5 min., 10% Fetal Bovine Serum (FBS) was

10   added to deactivate the trypsin, and the cell suspension was centrifuged at 1000RPM. The supernatant was aspirated and the cell pellet was resuspended in a proliferation media consisting of Keratinocyte-Serum Free Medium (KSFM)(Gibco-BRL, Grand Island, NY) containing 5ng/ml EGF (Gibco), 50μg/ml BPE (Gibco), 100 U/ml penicillin, 0.1mg/ml streptomycin, and 2mM L-glutamine. The cell suspension was transferred to 2,

15   10cm tissue culture dishes coated with 1μg/cm^2 Vitrogen (Becton-Dickinson, Bedford, MA), incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The cells were allowed to grow for 6 days (70-80% confluence) with the media being replaced with fresh media every other day. The cells were then trypsinized using 0.025% trypsin-EDTA for 5 min. The cell suspension was collected, the trypsin deactivated with 10%

20   FBS, and centrifuged at 1000 rpm for 5min. The cells were then counted using a hemocytometer. There was a typical yield of 3x10^6 cells per dish. The supernatant was aspirated and the cells were resuspended in KSFM and plated on 1μg/cm^2 Vitrogen at a density of 5x10^3 cells/cm^2.

CFHNE and HNE Cell Preparation: The epithelial cells (Passages 2 or 3) were

25   prepared for Ussing Chamber and fluid transport studies using Snapwell permeable supports (0.4 μm pore size; Corning Costar, Cambridge, MA) coated with 1 μg/cm^2 Vitrogen. Cells were plated at 10^5 cells/cm^2 in KSFM. After 2 days, the media was changed to BEGM (a 1:1 mixture of DMEM (MediaTech/Cellgro, Herndon, VA) and BEBM (Clonetics/Biowhittaker, Walkersville, MD), with the following supplements:

30   hydrocortisone (0.5 μg/ml), insulin (5 μg/ml), transferrin (10 μg/ml), epinephrine (0.5 μg/ml), triiodothyronine (6.5ng/ml), Bovine Pituitary Extract (52 μg/ml), EGF (0.5 ng/ml), All-trans retinoic acid (50 nM, Sigma), penicillin (100 U/ml, Sigma), streptomycin (0.1mg /ml, Sigma), non-essential amino acids (1X, Sigma), and Bovine

Serum Albumin (fatty acid-free, 3 µg/ml, Sigma). Media supplements were from Clonetics unless otherwise indicated. The CFHNE cells were grown in the BEGM for 1 week, at which point an air-liquid interface (ALI) culture system was initiated. The cells were grown for an additional 2 weeks at ALI, being fed every other day basolaterally,

5 until use in the Ussing chamber. Monolayers used for experiments were routinely fed the day before use.

Ussing Chamber Studies Monolayers of CFHNE were mounted in modified Ussing chambers (Physiologic Instruments, Palo Alto, CA) using Ringers bicarbonate solution containing (in mM): 115 NaCl, 2.4 K_2HPO_4, 0.4 KH_2PO_4, 1.2 $MgCl_2$, 1.2

10 $CaCl_2$, 25 $NaHCO_3$, 10 glucose; unless otherwise indicated. Experiments were carried out at 37°C and the pH adjusted to 7.4 by gassing with 95%O_2/5%CO_2. After an open-circuit equilibration period of ten minutes, the transepithelial potential difference was recorded and the cells subsequently voltage clamped at 0mV. The resulting current was continuously monitored. A periodic bipolar voltage pulse was introduced and the

15 resulting resistance calculated using Ohm's Law.

Acute effects of INO-4997, INO-4996 and INO-4984 (synthesized by Sichem GmbH, Bremen, Germany) on basal amiloride inhibitable I_{sc} were determined in accordance with the foregoing procedure. Figures 1 and 2 demonstrate the effects of INO-4996 and INO-4984 on basal spontaneous short circuit (Isc) current in cystic fibrosis

20 human nasal epithelia. Monolayers were cultured as described in methods and mounted in Ussing chambers for testing. Compounds were added directly to the apical compartment at the indicated times. Controls received vehicle concurrently.

Fig. 3 demonstrates the prolonged effect of a 2 hour pretreatment with INO-4997 on basal Isc measured 22 hours later.

25 EXAMPLE 2.

Blue Dextran Volume Transport Assay

In normal human airway epithelia, Na+ and Cl- currents (CFTR and Ca2+-activated Cl- currents) contribute to airway surface liquid (ASL) fluid volume regulation depending on signaling equilibria. In contrast, in human CF airway epithelia, Na+

30 currents through ENaC dominate basal ASL volume regulation accompanied by a relatively minor contribution through Ca2+-activated Cl- currents. The combination of enhanced ENaC currents and transient Ca2+-activated Cl- currents in CF result in an inadequate hydration of the ASL and reduction of mucociliary clearance. To demonstrate

the ability of the compounds of the invention to inhibit fluid absorption, well differentiated monolayer cultures of CF nasal epithelia were exposed to an apically applied buffer containing the compounds and a known concentration of the non-permeable molecule Blue Dextran (BD). The resulting reduction in the ability of these monolayers to concentrate BD was taken as a functional indicator of the test compound's involvement in the inhibition of ENaC.

All procedures were performed aseptically. The Blue Dextran (BD) stock solution was prepared with HEPES modified Ringer's buffer (HMRB) (2mg BD/ml buffer). The compounds tested were solubilized in HMRB containing ~1µM BD. Final concentration of vehicle is 0.1% unless otherwise indicated (1:1, DMSO+DMSO containing 5% (w/v) pluronic-F127). The composition of HMRB (pH 7.3, 6.7 when equilibrated with 95%air/5%CO_2) is as follows (in mM): 135 NaCl, 1.2 $CaCl_2$, 1.2 $MgCl_2$, 2.4 K_2HPO_4, 0.6 KH_2PO_4, 10 HEPES, 10 glucose (~285mOsm.). 200µl of the BD solution was applied to the apical surface and placed in a highly humidified incubator (Forma model 3956 set on "high" humidity) for 18 hours. Basolateral buffer consisted of BEGM (~300mOsm.). After 18 hours, 60µl of the remaining apical buffer was sampled and transferred to a 0.7ml micro-centrifuge tube for storage until analysis. A standard concentration curve was obtained by determining the optical densities, at 660nm, of a serial dilution of 10µM Blue Dextran in HMRB in a 96-well plate using a Packard Spectracount. The [BD] of the samples, which were read on the same plate, was determined by extrapolation from the BD standard curve using Packard I-Smart software. The increase in [BD] from the starting value of 1µM was taken to be an indication of the magnitude of volume absorption occurring across the monolayer. The rate of absorption was calculated by dividing the change in volume by the duration of the experiment. This value was normalized to a surface area of 1cm2, to give µl•cm2 -1•hr -1. All experiments, unless otherwise indicated, were conducted over a period of 18 hours. Evaporative loss did not contribute significantly to the data using this system.

Amiloride Dose-Dependently inhibits fluid absorption using the Blue Dextran Assay.

The basal absorption rate using the Blue Dextran Assay ranged between 4 and 6 µl/cm2, consistent with values for CF tissue reported in the literature (Jiang, et al., 1993 Science, 262 p424-427). To assess the ability of the Blue Dextran Assay to measure

relevant changes in fluid secretion, the effect of the sodium channel blocker amiloride was tested. A large component of the abnormal fluid absorption in CF is due to accelerated sodium absorption and blocking the sodium channel with amiloride would be expected to significantly reduce fluid absorption. As expected, amiloride inhibited fluid

5   absorption measured by the Blue Dextran assay in a dose dependent fashion. Therefore, this assay is suitable for evaluating the therapeutic potential of other compounds that inhibit sodium channels, such as inositol polyphosphate analogs.

The following compounds were tested in accordance with the foregoing procedure:

10   Table 1

Test Compounds

Compound ID No.	Compound	Figure
4984	2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester	5
4997	2-O- butyryl-1-O-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester	6
4996	1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester	7

The dose response analysis of the effects of a series of inositol polyphosphate analogs on fluid absorption in human CF nasal epithelia using the blue dextran (BD)

15   assay are shown in Figures 5-7. In these Figures, rates are compared with absorption rates in the presence of amiloride. Data are shown as means +/- SEM in the bar graphs of Figures 5-7.

These data show the effects of the tested inositol polyphosphate analogs on the inhibition of the average fluid absorption rate in human CF nasal airway epithelia. For

20   comparison, 100μM amiloride, a ligand that binds the apical sodium channel (ENaC) acutely inhibits fluid absorption, was included for comparison.

EXAMPLE 3.

Inhibition of iNOS by inositol polyphosphate analogs

The reactive product, nitric oxide (NO), of the inducible form of nitric oxide

25   synthase (iNOS) is a common component of inflammatory disease. This moiety acts as

an adjuvant for microbicidal activity and as an autocrine/paracrine cytokine. In chronic inflammatory disease, NO may be increased 100 fold. Normal levels of NO, the result of the action of cNOS or nNOS (the constitutively expressed isoforms) are in the picomole range whereas stimulated production (iNOS) is 1000 fold higher and can be sustained for

5   long periods. iNOS stimulation can result from bacterial products such as endotoxin or by inflammatory cytokines interferon, TNFα and IL-1 (see review, Ketteler, et. al., "Cytokines and L-arginine in renal injury and repair," *Am J Physiol Renal Physiol* 267:F197-F207 (1994)).

The value of using iNOS as a reporter for anti-inflammatory activity is in its

10   context of activation. The molecule may contribute to the rapid rise in reactive oxygen species (complexed with O_2^- to form peroxynitrite, OH⁻ and nitrogen dioxide) or suppress superoxide production. Peroxynitrite has been shown to induce IBD symptoms when infused rectally in rats. iNOS may figure in the character and progression of inflammation through modulation by cytokines (TGF-βand IL-12) and stimuli such as LPS. The NO

15   product can interfere with iron containing enzymes (electron transport) or activate poly(ADP-Ribose) synthetase, depleting cellular b-nicotinimide adenine dinucleotide and progressing to cell death.

We chose the well characterized LPS stimulation of the murine macrophage cell line RAW 264.7, that produces substantial quantities of NO, to screen inositol

20   polyphosphate compounds *in vitro* (Figures 8-12 and Table 1). This assay system offers a number of opportunities to characterize drug action as pre-transcriptional, post transcriptional, translational or post translational.

In addition to its role in inflammation, NO plays a role in radiation response. Referring to Figure 13, inositol signaling pathways are interwoven with radiation

25   exposure pathways regulating apoptosis and DNA repair. As used in Figure 13, pointed arrows denote positive regulation; blunted arrows, negative regulation. Pathway structure provides the opportunity for extensive cross talk and feedback. Phosphorylation of p53 on serine 15 (which can occur via ATM triggered by Ionizing Radiation (IR) or ATR triggered by UVB) interferes with MDM2 binding and ubiquitination of p53. NO down

30   regulates MDM2 but prolonged NO exposure results in MDM-resistant p53. Abbreviations shown in Figure 13 are as follows: PIP_3: Phosphatidylinositol 3,4,5, trisphosphate; IP_4: inositol 1,4,5,6- tetrakisphosphate; PI 3-K: phosphatidylinositol 3 kinase; ATM: ataxia telangiecstasia mutated gene product; MDM2, mouse double minute

2; P21: p21/Cip/WAF1; IP6K2: inositol hexakisphosphate kinase 2; ATR: NO: Nitric Oxide.

Role of Nitric Oxide (NO) in the radiation response. Ionizing Radiation (IR) potentiated inducible nitric oxide synthase (iNOS) induction by LPS in murine macrophages (McKinney et al., "Ionizing radiation potentiates the induction of nitric oxide synthase by interferon-gamma and/or lipopolysaccharide in murine macrophage cell lines. Role of tumor necrosis factor-alpha," *Ann N Y Acad Sci* **899**:61-68 (2000)) while ultraviolet radiation (UV) stimulates nitric oxide (NO) production in keratinocytes (Romero-Graillet et al., "Nitric oxide produced by ultraviolet-irradiated keratinocytes stimulates melanogenesis," *J Clin Invest* **99**:635-42 (1997)). NO production is inhibited by the phosphatidylinositol 3- kinase/Akt/PKB pathway(Wright and Ward, 2000). NO, in turn, down regulates MDM2 protein but not mRNA levels resulting in elevation of p53 and p21 Cip/WAF1 levels (Hofseth et al., "Nitric oxide-induced cellular stress and p53 activation in chronic inflammation," *Proc Natl Acad Sci U S A* **100**:143-148 (2003)). This appears to be the primary mechanism for NO regulation of p53 since NO signaling to p53 does not require ATM poly (ADP-ribose) polymerase 1 (Wang et al., "p53 Activation by nitric oxide involves down-regulation of Mdm2," *J Biol Chem* **277**:15697-15702 (2002)). This is consistent with the findings of (Phoa and Epe, "Influence of nitric oxide on the generation and repair of oxidative DNA damage in mammalian cells," *Carcinogenesis* **23**:469-475 (2002)) who demonstrated that endogenous NO production in fibroblasts was associated with protection from DNA strand breaks. This suggests that the PI 3-K dependent reversal of UV irradiation-mediated suppression of p21 in insulin-like growth factor 1 (IGF-1) stimulated cells could be mediated by regulation of NO production. (Murray et al., "IGF-1 activates p21 to inhibit UV-induced cell death," *Oncogene* **22**:1703-11 (2003)). Therefore, regulation of NO production could provide benefit in the treatment of exposure to ultraviolet or ionizing radiation or chemotherapeutic agents used in the treatment of hyperproliferative disorders such as cancer. NO production may also help inhibit cell proliferation in hyperproliferative disorders such as cancer, tumors, schleroderma, autoimmune disease, and hyperproliferative skin disorders such as psoriasis.

iNOS protocol

Determination of Nitrite Anion Production

Nitrite (NO_2^-) accumulation in cell-free supernatant, used as an indicator of NO production, was measured using the modified Greiss reagent (Sigma G4410, St Louis, MO). This method was applied as described elsewhere (Martinez, J. et al., "Regulation of prostaglandin E2 production by the superoxide radical and nitric oxide in mouse

5   peritoneal macrophages," *Free Radical Res* 32:303-311 (2000)). Briefly, RAW264.7 cells (ATCC, #TIB-71) were maintained in standard tissue culture flasks and with DMEM+10% heat inactivated fetal bovine serum(HI-FBS) (Fetal-bovine serum, Heat-inactivated, Sercare Life Sciences, Oceanside, CA). Experiments were initiated by seeding logarithmic cells at 2^5 per well in a 96-well plate in phenol-red free RPMI 1640

10   (Sigma #R8755)+10% HI-FBS at least four hours before the initiation of the experiment. Cells were exposed to treatment compounds for a minimum of 30 minutes in RPMI-1640 supplemented with 10% fetal calf serum. The compound treatment was removed and inflammatory compounds dissolved in RPMI-1640 supplemented with 10% FBS were applied to the cells. Stimulated cells and appropriate controls were incubated for 18-24

15   hours before sampling 50µl of the cell-free supernatant from each well. This sample of supernatant was combined with 25µl of a nitrate reductase cocktail (0.1 units/ml nitrate reductase enzyme, 5µM FAD, 30µM NADPH) and incubated for 30 minutes-37°C. To this mixture, 25µl of LDH cocktail (100units/ml rabbit muscle lactate dehydrogenase in 0.3mM sodium pyruvate) was added and the total mixture was incubated another 5-10

20   min at -37°C. Greiss reagent was added in 100µl amounts to each 100µl experimental sample. Color development proceeded for a minimum of 10 minutes while protected from light. Nitrite levels were compared to a sodium nitrite standard curve freshly prepared in the same medium used for the growth and incubation of the cells and treated with the same enzyme treatments. Color development was recorded by using a Packard

25   Spectracount Plate reader at 540nm wave length.

Effects of INO-4996, INO-4984, and INO-4997 on iNOS induction in a macrophage cell line. We identified a number of molecules which showed a suppression of NO (as $NaNO_2$), over a concentration gradient of 300 nM to 100 mM over 17-24 hrs continuous exposure as seen (Figs. 8-12). The LPS stimulation, 100 nM with 100 mM

30   ATP, was intentionally sub maximal to allow for the discovery of compounds which might enhance iNOS activity. IC_{50}'s for compounds ranged from 5–12 µM, and some compounds had little or no activity in this setting. Compound activity distributed around the character of the pro-drug protecting groups according to hydrophobicity. This

distribution is predictable from the relative permeability conferred by the respective protecting groups. Within this distribution another grouping was discerned; a correlation with the pattern of phosphate substitutions. In Figures 8-12, the effects of INO-4996, INO-4984 and INO-4997 on iNOS induction are contrasted with Dexamethasone.

It is important to identify the conditions under which the compounds exert their greatest desired effect with the minimal adverse effects. Understanding this relationship is important as signaling molecule mimetics may require special conditions of concentration and duration to generate the desired therapeutic effect. To ascertain whether effects could be observed post-exposure to stimulus, cells were exposed to the LPS/ATP stimulation for two hours at 37° C after which compound was added. The results of three experiments with a calculated IC_{50} for each compound are shown in the table below.

Table 1

Survey of iNOS Inhibition – 2 hr LPS pre-stimulation

Compound	IC_{50}	IC_{50}	IC_{50}
INO-4996	6.25 μM	3.13 μM	6.25 μM
INO-4984	18 μM	3.13 μM	12.5 μM
INO-4997	12.5 μM	12.5 μM	9.0 μM

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15   While the preferred embodiments of the invention have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1.   A sodium ion absorption and/or inducible nitric oxide synthase (iNOS) inhibiting camphanylidene or phenyl alkyl inositol polyphosphate compound, or a stereoisomer, racemate, prodrug or a pharmaceutically acceptable salt thereof.

2.   A camphanylidene inositol polyphosphate compound selected from the group consisting of 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate, 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate, and the stereoisomers, racemates, prodrugs, esters and pharmaceutically acceptable salts thereof.

3.   A compound of Claim 2, which is an ester selected from the group consisting of acetoxymethylesters (AM-esters), propionoxymethylesters (PM-esters) or pivaloyloxymethyl esters.

4.   A compound of Claim 3 selected from the group consisting of 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester, and 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester, and the stereoisomers, racemates, prodrugs, and pharmaceutically acceptable salts thereof.

5.   A method for inhibiting sodium ion absorption by epithelial cells, comprising treating the cells with an effective amount of a sodium uptake inhibiting camphanylidene and/or phenyl alkyl inositol polyphosphate compound.

6.   A method for inhibiting sodium ion absorption by epithelial cells in a human or animal patient in need of such treatment, comprising administering to the patient a therapeutically effective amount of a sodium uptake inhibiting camphanylidene and/or phenyl alkyl inositol polyphosphate compound.

7.   A method of Claim 6, wherein the sodium uptake inhibiting camphanylidene and/or phenyl alkyl inositol polyphosphate compound is a sodium uptake inhibiting inositol polyphosphate compound.

8.   A method of Claim 6, wherein the camphanylidene inositol polyphosphate compound is selected from the group consisting of 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate, 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate, and the stereoisomers, racemates, prodrugs, esters and a pharmaceutically acceptable salts thereof.

9.   A method of Claim 8, which is an ester selected from the group consisting of acetoxymethylesters (AM-esters), propionoxymethylesters (PM-esters) or pivaloyloxymethyl esters.

10.   A method of Claim 9 selected from the group consisting of 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester, and 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester, and the stereoisomers, racemates, prodrugs, and pharmaceutically acceptable salts thereof.

Abstract of the Disclosure

This invention relates to new camphanylidene and phenyl alkyl inositol polyphosphate derivatives that inhibit the absorption of sodium ions in epithelial cells and regulate inducible nitric oxide synthase (iNOS) in macrophages. The invention provides

5   methods for inhibiting sodium ion absorption by epithelial cells and/or regulating inducible nitric oxide synthase (iNOS) in macrophages, by treating epithelial cells or administering to a patient in need of such treatment a therapeutically effective amount of camphanylidene and/or phenyl alkyl inositol polyphosphate compound. Representative camphanylidene and phenyl alkyl inositol polyphosphate compounds include, for

10   example, 1,2-camphanylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4996), 2,3-camphanylidene-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4984) and 2-*O*-butyryl-1-*O*-(3-phenylpropyl)-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl) ester (INO-4997).

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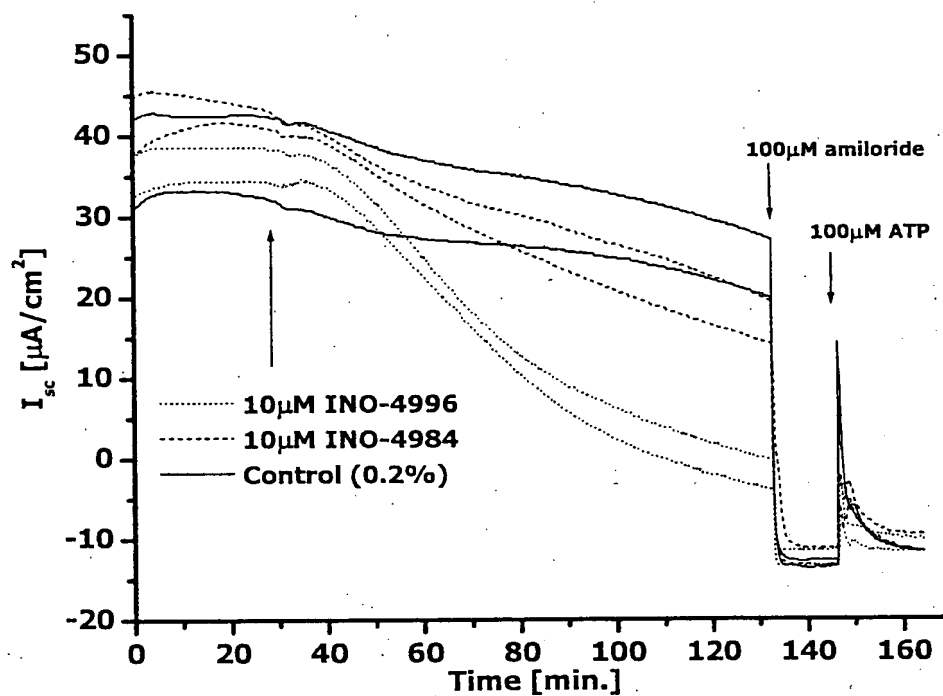
Fig. 1

Effect of acute exposure to 1 and 10μM 4997 on basal I_{sc} in CF human nasal epithelia, passage 3 (5CFHNEP3)

Fig. 2

Inhibition of Amiloride inhibitable I_{sc} 110 min.
following addtion of INO-4997

Fig. 3

Effect of INO-4997 in 5CFHNEP3 Monolayers.
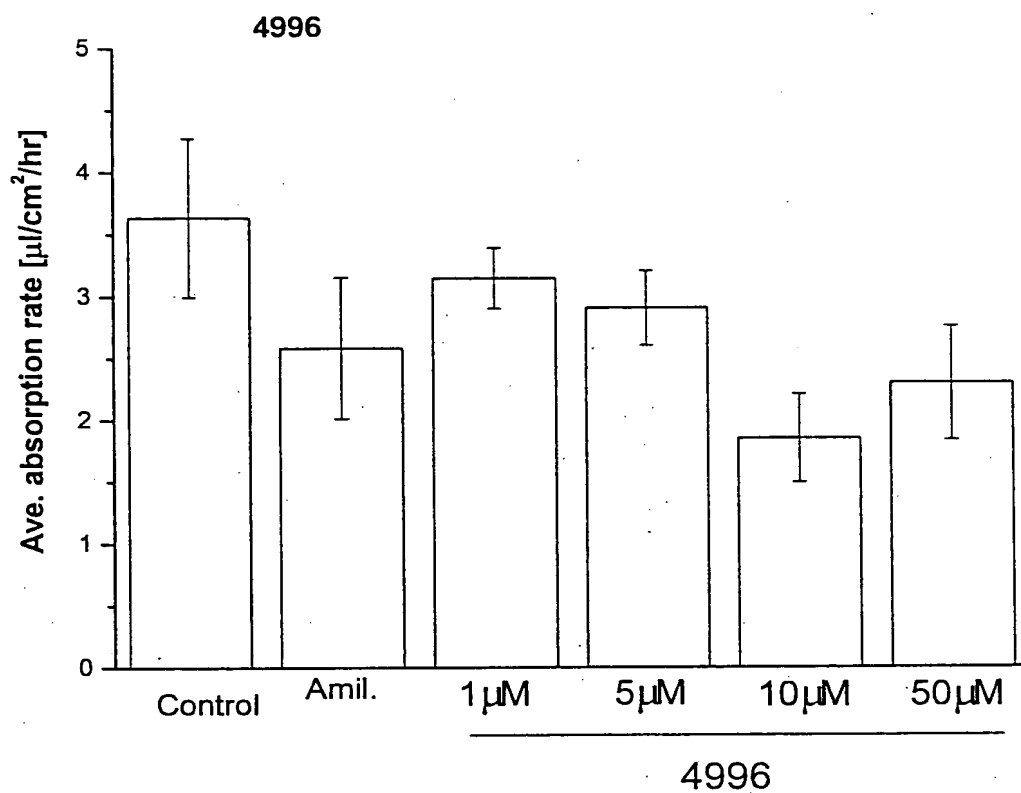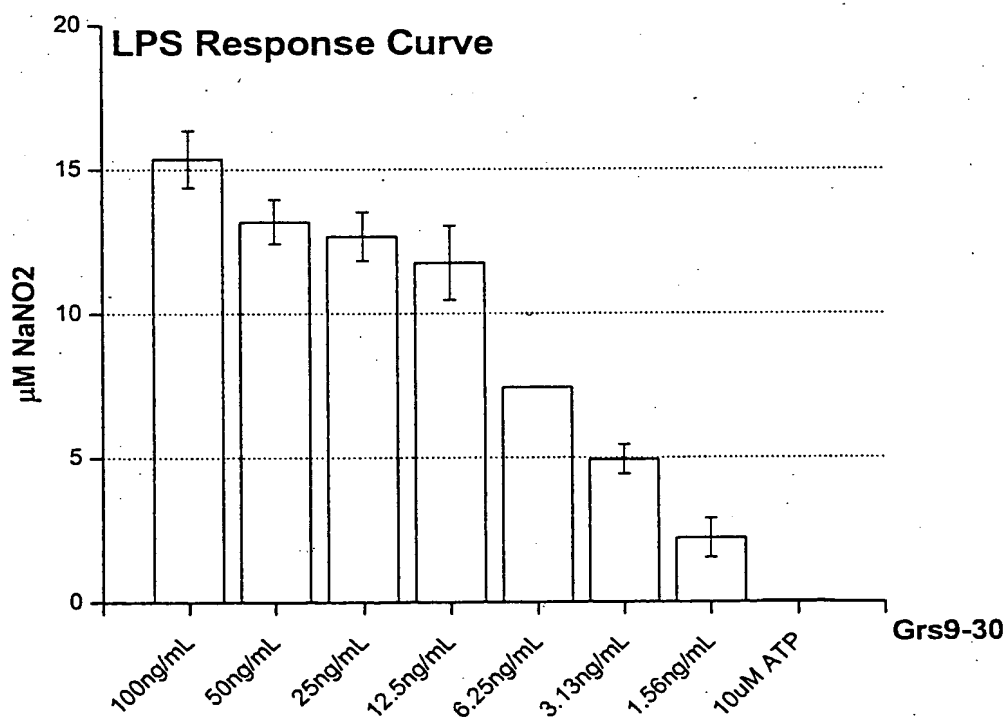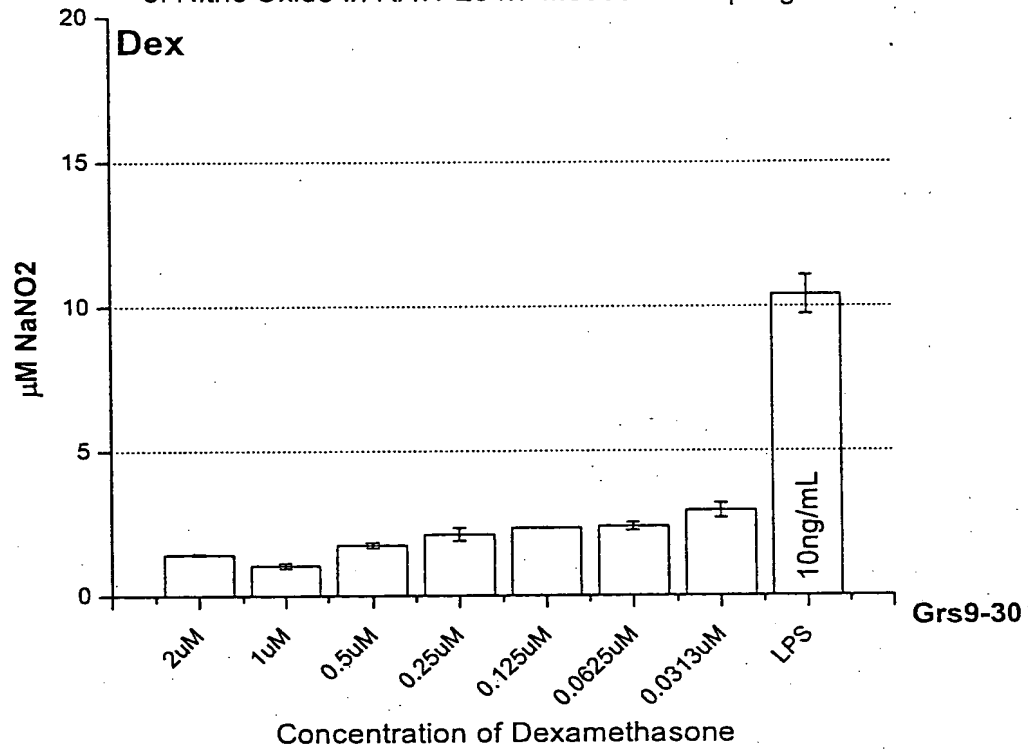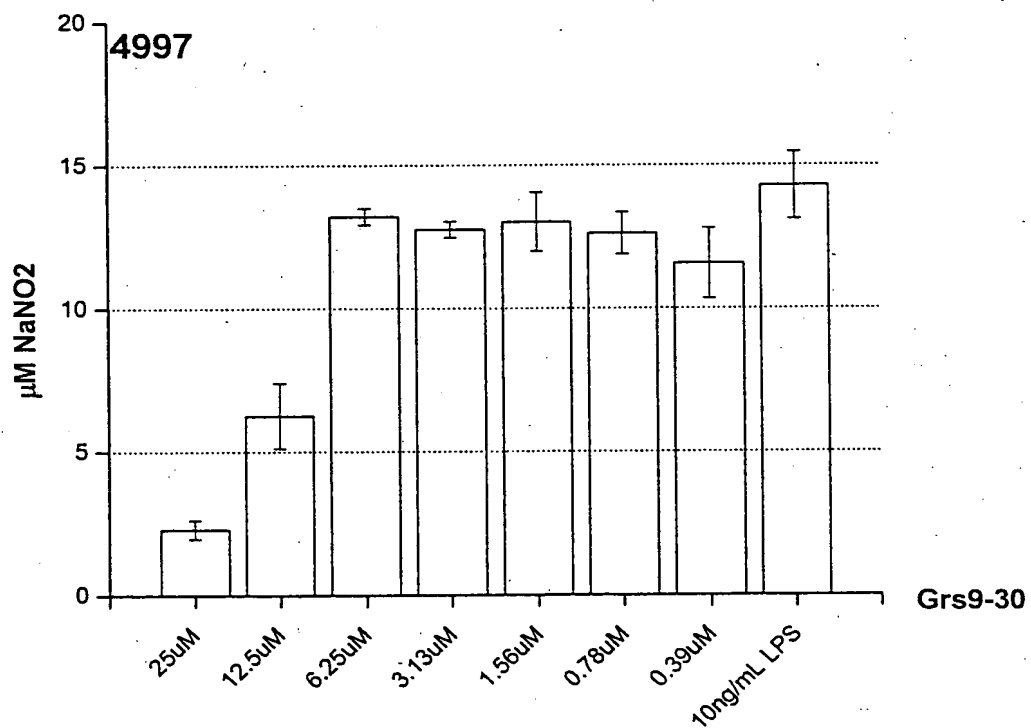

Fig. 4

Fig. 5

Fig. 6

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4996
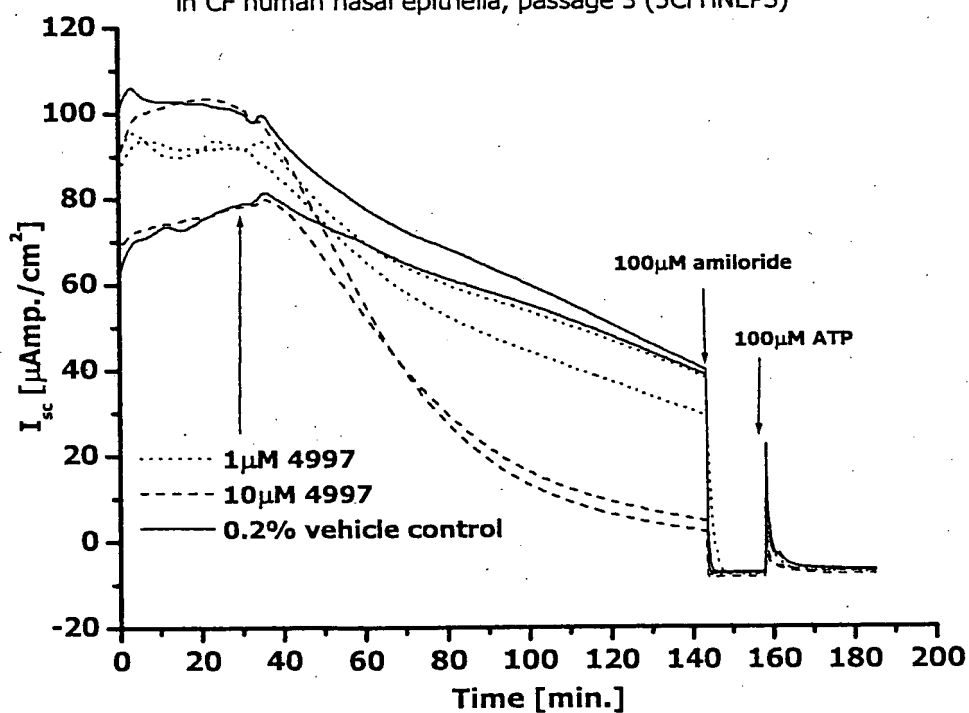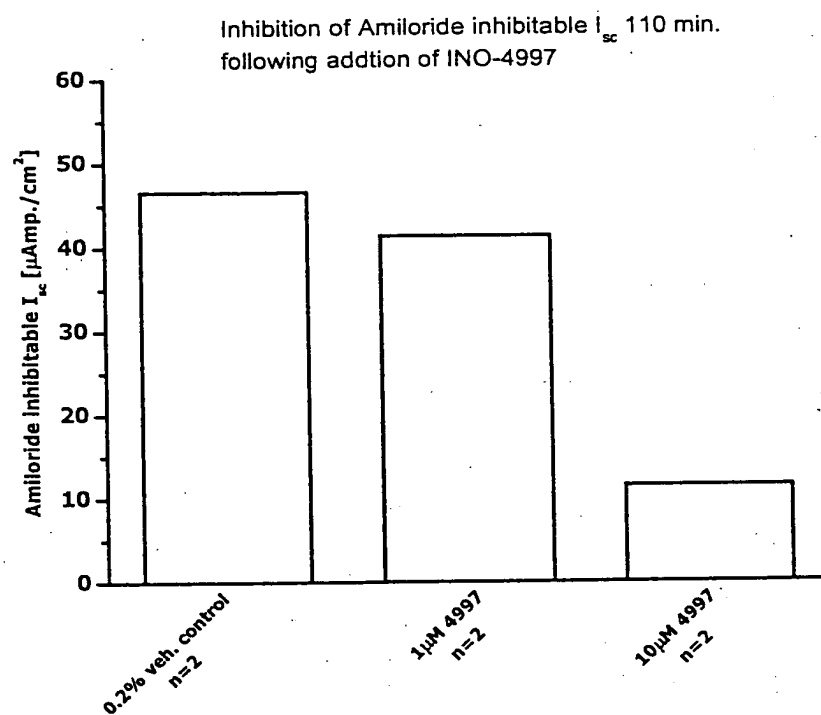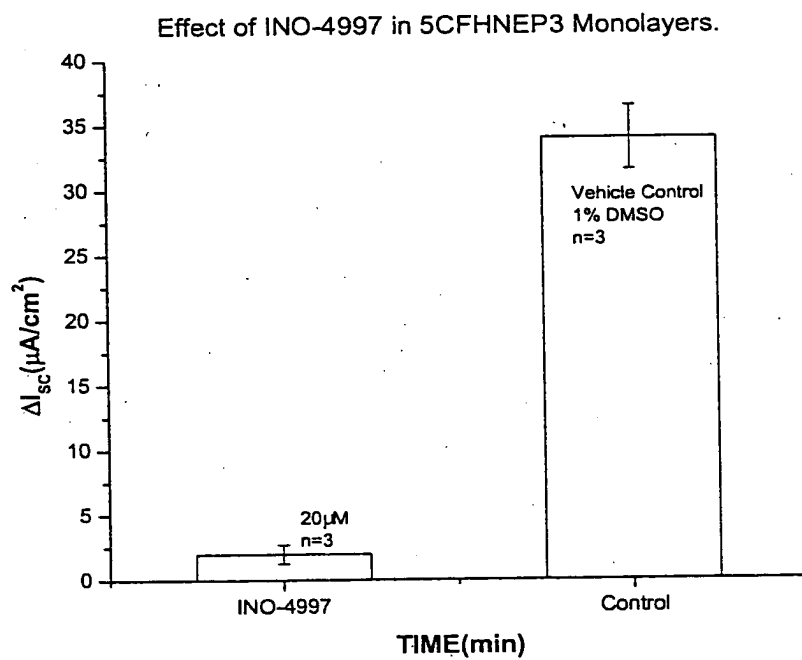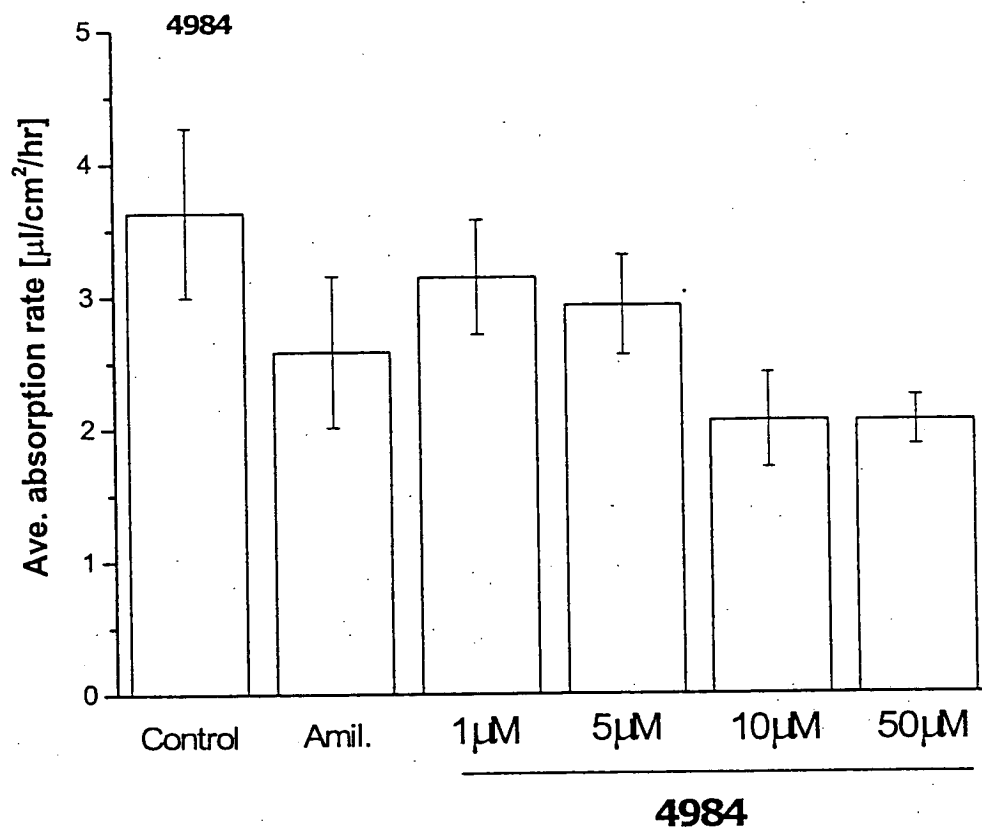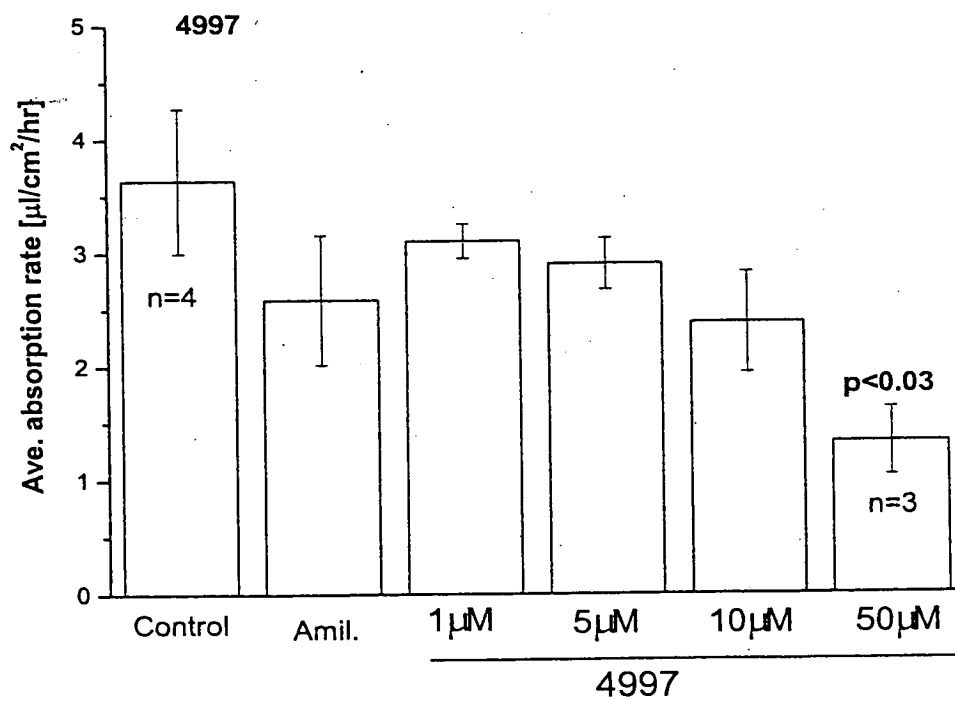
Fig. 7

Fig. 8

Dexamethasone, as Positive Control, Attenuates LPS Induction
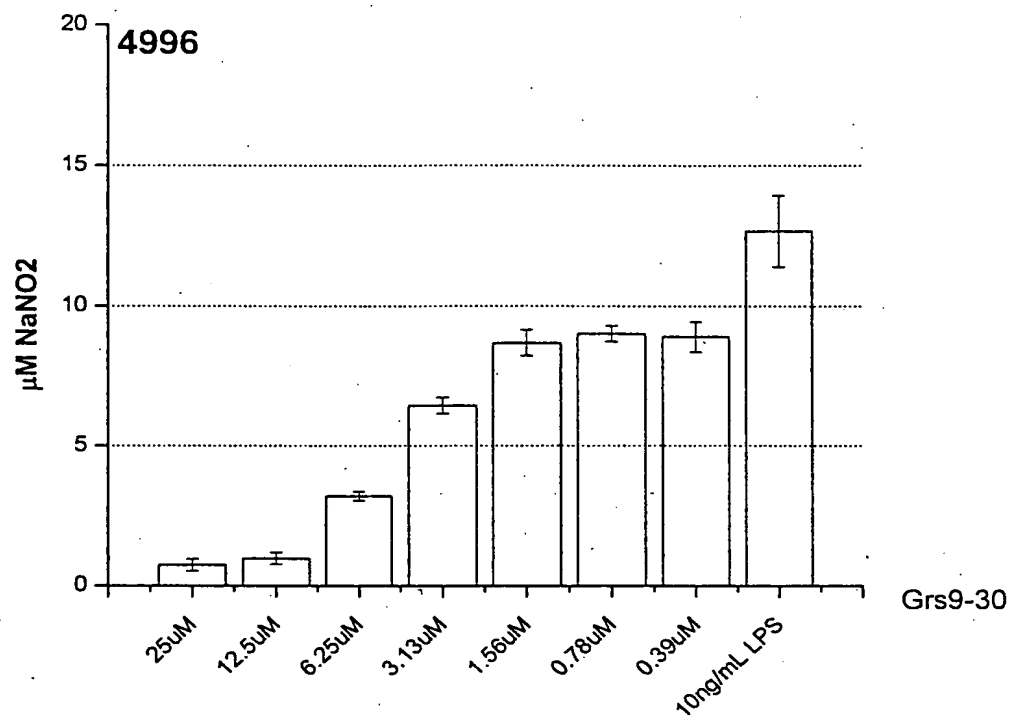of Nitric Oxide in RAW 264.7 Mouse Macrophages.

Fig. 9

Fig. 10

Fig. 11

Fig. 12
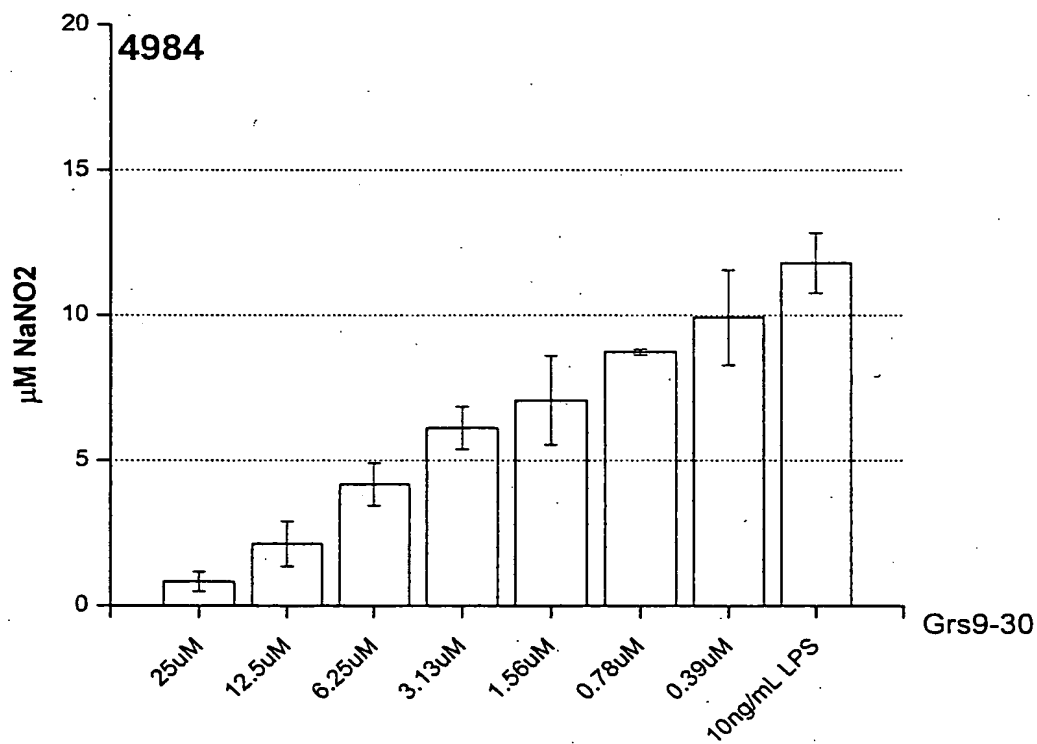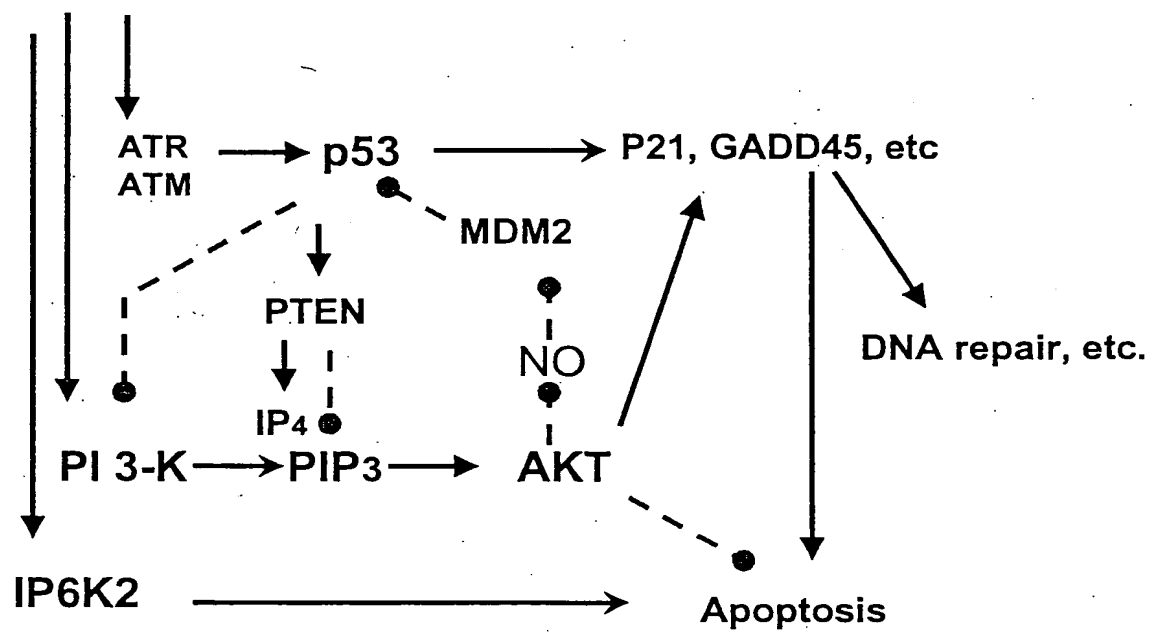
Fig. 13